Critical Reviews in Oncology / Hematology 136 (2019) 37–47

Contents lists available at ScienceDirect

Critical Reviews in Oncology / Hematology

journal homepage: www.elsevier.com/locate/critrevonc

Efficacy of granulocyte colony stimulating factor in combination with erythropoiesis stimulating agents for treatment of anemia in patients with lower risk myelodysplastic syndromes: A systematic review

Lucas Affentranger[a], Julia Bohlius[b], Mahmoud Hallal[a,c], Nicolas Bonadies[a,c,*]

[a] Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Switzerland
[b] Institute of Social and Preventive Medicine, University of Bern, Switzerland
[c] Department for BioMedical Research, University of Bern, Switzerland

ARTICLE INFO

Keywords:
Myelodysplastic syndromes
Erythropoiesis stimulating agents
Granulocyte colony stimulating factor
Erythroid response
Systematic review

ABSTRACT

Anemic patients with lower risk myelodysplastic syndromes are frequently treated with erythropoiesis stimulating agents (ESA), eventually in combination with granulocyte colony stimulating factor (G-CSF). However, the evidence for the efficacy of a combined treatment remains controversial. The goal of our analysis was to assess the available evidence for a combined treatment. We performed a systematic review and identified only nine eligible studies. In two randomized controlled trials (n = 98), erythroid response rates were 33% and 40% after low-/standard-doses of ESA alone (10,000-30,000 rHuEPO equivalents/week) versus 65% and 73% after combination treatment. In seven trials with sequential drug administration (n = 393), erythroid response rates ranged from 12% to 71% after full-doses of ESA alone (60,000-80,000 rHuEPO equivalents/week) and from 35% to 74% after combination therapy. Our analysis supports an additional efficacy of G-CSF added to low-/standard-dose ESA, but the available data remains controversial, if G-CSF is added to full-dose ESA.

1. Introduction

Myelodysplastic syndromes (MDS) are heterogeneous bone marrow diseases characterized by dysplasia and uni- or multilineage cytopenia. Anemia is the most commonly observed cytopenia and affected patients present with symptoms like fatigue, weakness, dyspnea and cardiovascular events, although some patients may be asymptomatic at diagnosis. The risk of progression to acute myeloid leukemia (AML) depends on the number of lineages affected by cytopenia, cytogenetic alterations and blast-count (Steensma, 2015; Greenberg et al., 1997, 2012). The age-standardized incidence-rate of MDS ranges from 4 to 5 cases per 100,000 person-years, whereas the age-specific incidence-rate in patients after the age of 65 years increases steadily over 50 cases per 100,000 person-years (Cogle, 2015; Bonadies et al., 2017). Indeed, aging is the most relevant risk factor for the development of MDS, whereby 85% to 90% of MDS cases are primary and relate to aging-associated, genetic alterations of the hematopoietic stem cells (Steensma, 2015).

Diagnostic classification of MDS is based on the *World Health Organization* criteria published in 2001 and revised in 2008 as well as 2016 (Jaffe et al., 2001; Swerdlow et al., 2008, 2016). Prognostic classification relies on the *International Prognostic Scoring System* (IPSS) (Greenberg et al., 1997) or revised IPSS (IPSS-R) (Greenberg et al., 2012), which are relevant for selection of appropriate treatment. Diagnosis of MDS subtypes and disease-based risk stratification require information on the number of cell-lines affected by cytopenia and dysplasia, percentage of blasts in peripheral blood and bone-marrow, ring sideroblasts, cytogenetic evaluation and more recently also assessment of somatic mutations of myeloid driver genes (e.g. *SF3B1*).

According to treatment recommendations from *European Leukemia Net* (Malcovati et al., 2013) and the *National Comprehensive Cancer Network* (Greenberg et al., 2017), preferred treatment for eligible higher risk MDS patients (IPSS intermediate-2 and high risk) consists of intensive chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT). However, only a minority of high-risk MDS

Abbreviations: AML, Acute myeloid leukemia; CI, Confidence interval; EPO, Erythropoietin; ESA, Erythropoiesis stimulating agents; G-CSF, Granulocyte colony stimulating factor; GM-CSF, Granulocyte-macrophage colony stimulating factor; HSCT, Hematopoietic stem cell transplantation; IPSS, International prognostic scoring system; IPSS-R, Revised-IPSS; IWGc, International working group criteria; MDS, Myelodysplastic syndromes; rHuEPO, Recombinant human erythropoietin; RBC, Red blood cell; RCTs, Randomized controlled trials; SDADs, Trials with sequential drug administration design

* Corresponding author at: Department of Hematology and Central Hematology Laboratory, Department of BioMedical Research, Inselspital, Bern University Hospital, University of Bern, CH-3010 Bern, Switzerland.
E-mail address: Nicolas.Bonadies@insel.ch (N. Bonadies).

https://doi.org/10.1016/j.critrevonc.2019.01.021
Received 9 October 2018; Accepted 26 January 2019

patients are fit enough for this intensive and potentially curative approach. Non-eligible patients receive palliative treatment consisting of hypomethylating agents that can improve peripheral cell-counts and prolong survival (Fenaux et al., 2009). Lower risk MDS (IPSS low risk and intermediate-1) is characterized by prolonged survival and lower risk of progression to AML. Thus, in this patient population, the focus is mainly on symptomatic therapy to improve quality of life and reduce progression to higher risk MDS or AML. For therapy of anemia, the current recommendations suggest supportive care including red blood cell (RBC) transfusions, iron-chelation and the application of growth-factors. For specific MDS subtypes, immunomodulatory treatment like antithymocyte globulin or cyclosporine in hypoplastic MDS or lenalidomide in MDS with del(5q) syndrome can be recommended. In patients with low transfusion burden (< 2 RBC units per month) and serum-EPO < 500 IU/L, current guidelines provide a strong recommendation for the administration of erythropoiesis stimulating agents (ESA, e.g. epoetin alpha, epoetin beta or darbepoetin) in combination with granulocyte colony stimulating factor (G-CSF) (Malcovati et al., 2013; Greenberg et al., 2017).

A synergistic effect of G-CSF in combination with ESA was found in experimental model systems and seems to be related to decreased cytochrome C release, which results in inhibition of apoptosis during erythroid differentiation (Hogge et al., 1991; Tehranchi et al., 2005; Chen et al., 2018). The application of G-CSF in combination with ESA is established in clinical treatment of anemia in lower risk MDS patients. However, the degree of evidence derived from properly randomized controlled trials (RCTs) investigating the additional effect of ESA + G-CSF compared to ESA alone remains unclear. Here we provide a systematic review of clinical trials that investigated the erythroid response rates in anemic lower risk MDS patients treated with ESA + G-CSF compared to ESA alone.

2. Methods

2.1. Criteria for selection of eligible studies

We initially focused on RCTs investigating the effect of ESA + G-CSF or granulocyte-macrophage colony stimulating factor (GM-CSF) versus ESA alone in anemic lower risk MDS patients. Due to the low number of RCTs with adequate design for our study question, we included also non-randomized, clinical trials with sequential drug administration design (SDAD) investigating the effect of G-CSF or GM-CSF in combination with ESA after minor or no erythroid response to ESA alone. Primary endpoint of interest was erythroid response according to *International Working Group criteria* (IWGc) (Cheson et al., 2000) or revised IWGc (Cheson et al., 2006). Clinical trials with erythroid response criteria similar to IWGc were evaluated individually for inclusion. Secondary endpoints were overall survival, progression-free survival, progression to AML, quality of life and severe adverse events.

2.2. Literature search

A systematic search in *Cochrane Library*, *Embase* and *PubMed* as well as conference databases from the *American Society of Hematology* and the *European Hematology Association* was performed for interventional studies. Detailed search strategies are shown in supplementary Table 1. No language or time restriction were applied to reduce the risk of missing relevant articles. Moreover, the following databases were searched for ongoing trials: clinicaltrials.gov (https://clinicaltrials.gov/), EU clinical trials register (https://www.clinicaltrialsregister.eu/ctr-search/search), International Clinical Trials Registry Platform (http://apps.who.int/trialsearch/) and Metaregister of Controlled Trials (http://www.controlled-trials.com/mrct/). The initial literature search was performed on January 2nd, 2018 and was repeated on June 10th, 2018 without identifying any additional relevant study. Reference sections from eligible trials and from recent reviews were manually checked for additional eligible studies.

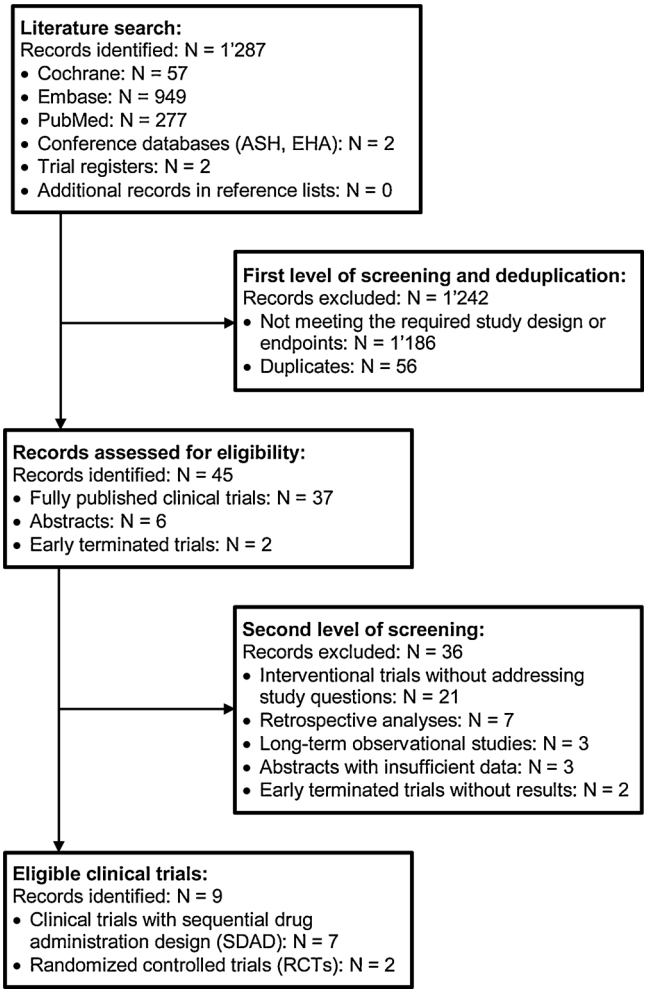

Fig. 1. Flow diagram of the systematic literature search and the study selection process according to *PRISMA* guidelines (Moher et al., 2009). Abbreviations: ASH, American Society of Hematology; EHA, European Hematology Association.

2.3. Study selection

All identified records were imported in Endnote 7 for the selection process. After deduplication, one review author (LA) evaluated titles and abstracts and excluded all records that were clearly ineligible. The eligible studies were reviewed independently by two additional authors (JB, NB). Full-text publications were retrieved, and eligibility was systematically assessed by using a standardized form (Supplementary Fig. 1). Observational and retrospective studies, reviews, letters, case reports and editorials were excluded. Any doubtful article for inclusion was discussed in the group of authors or by consulting another reviewer. The study selection process is shown in a flow diagram according to *PRISMA* (Moher et al., 2009) (Fig. 1).

2.4. Data collection, extraction and analysis

Data from selected studies was extracted by one author (LA) and integrated into a previously prepared and piloted data extraction form (Supplementary Table 2). Data extraction was verified by a second

author (NB) by double-checking. Disagreement was again resolved by discussion in the author group or by consulting another reviewer. OpenMeta[Analyst] software (http://www.cebm.brown.edu/openmeta/) was used to summarize data from included trials. Relative risks with 95% confidence intervals (CI) are shown for RCTs and proportions of erythroid response with 95% CI for clinical trials with SDAD, respectively. Summaries were calculated by using a random effects model.

2.5. Assessment of risk of bias

Risk of bias for RCTs and non-RCTs was determined by using the predefined criteria of the *Cochrane Collaboration* for assessing risk of bias in randomized trials (Higgins et al., 2011) and in non-randomized trials (Sterne et al., 2016).

3. Results

3.1. Screening for eligible studies

In total, 1,287 references were identified in our literature search. Fifty-six records were excluded after deduplication and another 1,186 after first level of title and abstract screening due to unsuitable study design or endpoints (total 1,242). The full-text publications of the remaining and potentially eligible 45 records were retrieved. After the second level of screening, nine records met the specified eligibility criteria. All eligible records were written in English and published between 1998 and 2013. Two additional, unpublished studies were identified in the trial registers (NCT00234143, NCT01196715), which would have generally met our eligibility criteria. However, these studies were terminated prematurely without publishing results and our requests for further information remained unanswered.

3.2. Designs of eligible studies

Two of the nine records were RCTs with adequate design for our study question, comprising totally 98 patients (Balleari et al., 2006; Nair et al., 2019), and one of these studies was a conference abstract (Nair et al., 2019) (Table 1). Seven studies were clinical trials with SDAD investigating the erythroid response in 393 patients (Hellstrom-Lindberg et al., 1998; Remacha et al., 1999; Mannone et al., 2006; Gotlib et al., 2009; Greenberg et al., 2009; Villegas et al., 2011; Kelaidi et al., 2013) (Table 2). Five of these studies were single-arm phase 2 prospective trials (n = 257) (Remacha et al., 1999; Mannone et al., 2006; Gotlib et al., 2009; Villegas et al., 2011; Kelaidi et al., 2013) and two studies were RCTs (n = 136) (Hellstrom-Lindberg et al., 1998; Greenberg et al., 2009), where the randomized interventions in these two RCTs did not match our research question. One of these RCTs investigated the effect of recombinant human erythropoietin (rHuEPO) followed by rHuEPO + G-CSF in one arm compared to the effect of G-CSF alone followed by rHuEPO + G-CSF in the other arm (Hellstrom-Lindberg et al., 1998). The other RCT investigated the efficacy of rHuEPO alone followed by rHuEPO + G-CSF in non-responders in one arm compared to supportive care only in the comparator arm (Greenberg et al., 2009). From these studies, we extracted data from one study arm only, in which ESA treatment was followed by the combination of ESA + G-CSF, which matched our inclusion criteria as clinical trials with SDAD. There was no suitable publication investigating the additional effect of ESA + GM-CSF in MDS patients. One potentially eligible study investigating GM-CSF from Bernell and colleagues was excluded due to treatment interruptions and unsuitable sequence of drug administration (Bernell et al., 1996). In summary, we were able to include nine clinical trials with a total of 491 MDS patients, of which 98 patients were included in two properly designed RCTs and 393 patients in seven studies with SDAD.

Table 1
Patient characteristics of RCTs.

Study *Author (Year)*	Patients evaluated *n*	FAB *n (%)*	WHO 2008 *n (%)*	IPSS *n (%)*	Hemoglobin *g/L*	Required Serum-EPO level for inclusion *IU/L*	Serum-EPO *IU/L*	Transfusion-dependency *n (%)*	Nordic MDS group score *n (%)*
(Balleari et al., 2006)	30	N/A	RA: 10 (33) RARS: 5 (17) RCMD: 7 (23) RAEB-1: 5 (17) 5q: 3 (10)	Low: 10 (33) Int-1: 20 (67)	Median: 85 Range: 70-100	N/A	N/A	11 (37)	N/A
(Nair et al., 2019)	68	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Abbreviations: FAB, French-American-British; Int, intermediate; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndromes; N/A, not available; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenias with multilineage dysplasia; WHO 2008, World Health Organization Classification 2008; 5q, del(5q) syndrome.

Table 2
Patient characteristics of clinical trials with SDAD.

Study *Author (Year)*	Patients evaluated *n*	FAB *n (%)*	WHO 2008 *n (%)*	IPSS *n (%)*	Hemoglobin *g/L*	Required Serum-EPO level for inclusion *IU/L*	Serum-EPO *IU/L*	Transfusion-dependency *n (%)*	Nordic MDS group score *n (%)*
(Hellstrom-Lindberg et al., 1998)	26	RA: 6 (23) RARS: 5 (19) RAEB: 15 (58)	N/A	Low: 7 (27) Int-1: 9 (35) Int-2: 7 (27) Missing: 3 (11)	Mean: 86	N/A	Mean: 237 Range: 20-4'128	18 (69)	N/A
(Remacha et al., 1999)	32	RA: 9 (28) RARS: 23 (72)	N/A	N/A	Mean: 83 Range: 61-104	< 250	N/A	21 (66)	High: 18 (56) Int: 14 (44)
(Mannone et al., 2006)	62	RA: 22 (36) RARS: 20 (32) RAEB: 18 (29) CMML: 2 (3)	RA: 11 (18) RARS: 18 (29) RCMD: 8 (13) RCMD-RS: 2 (3) RAEB-1: 18 (29) 5q: 3 (5) CMML-1: 2 (3)	Low: 16 (26) Int-1: 26 (42) Int-2: 8 (13) Missing: 12 (19)	N/A	< 500	Median: 67 Range: 6-487	41 (66)	N/A
(Gotlib et al., 2009)	24	RA: 10 (41) RARS: 9 (38) RAEB: 3 (13) CMML: 2 (8)	RCMD: 8 (33) RCMD-RS: 9 (38) RAEB-1: 3 (13) 5q: 2 (8) CMML-1: 2 (8)	Low: 12 (50) Int-1: 10 (42) Int-2: 2 (8)	Median: 93 Mean: 92 Range: 71-108	N/A	Median: 111 Range: 12-2'556	16 (67)	High: 14 (58) Int: 9 (38) Low: 1 (4)
(Greenberg et al., 2009)	110	RA: 42 (38) RARS: 37 (34) RAEB: 29 (26) CMML: 2 (2)	N/A	Low / Int-1: 91 (83) Int-2 / High: 19 (17)	N/A	N/A	N/A	67 (61)	N/A
(Villegas et al., 2011)	44	RA: 14 (32) RARS: 27 (61) RAEB: 3 (7)	N/A	Low: 34 (77) Int-1: 10 (23)	Mean: 92	< 500	Mean: 121	12 (27)	N/A
(Kelaidi et al., 2013)	95	N/A	RA: 24 (25) RARS: 31 (33) RCMD: 15 (16) RCMD-RS: 7 (7) RAEB-1: 14 (15) 5q: 2 (2) CMML: 2 (2)	Low: 51 (54) Int-1: 44 (46)	Median: 92 Range: 62-100	< 500	Median: 60 Range: 3-461	44 (46)	N/A

Abbreviations: CMML, chronic myelomonocytic leukemia; FAB, French-American-British Classification; Int, intermediate; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndromes; N/A, not available; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenias with multilineage dysplasia; RCMD-RS, refractory cytopenias with multilineage dysplasia and ring sideroblasts; WHO 2008, World Health Organization Classifcation; 5q, del(5q) syndrome.

3.3. Patient characteristics

From all included patients, 62% were male and 38% were female with a mean age of 69 years (range of 24–91 years). Inclusion criteria reported for the selected studies included hemoglobin < 100 g/L or transfusion-dependency in four trials (Hellstrom-Lindberg et al., 1998; Remacha et al., 1999; Mannone et al., 2006; Gotlib et al., 2009), hemoglobin < 100 g/L in three trials (Balleari et al., 2006; Villegas et al., 2011; Kelaidi et al., 2013) and hemoglobin < 95 g/L in the study of Greenberg et al. (2009). Three trials with SDAD required for inclusion serum EPO baseline levels < 500 IU/L (Mannone et al., 2006; Villegas et al., 2011; Kelaidi et al., 2013) and one trial < 250 IU/L (Remacha et al., 1999). These four studies with SDAD focused, therefore, on populations with a higher chance of response. Percentage of transfusion-dependent patients included in the studies ranged from 27% to 70%. Numbers of RBC units transfused per month were only reported in four studies (Hellstrom-Lindberg et al., 1998; Remacha et al., 1999; Villegas et al., 2011; Kelaidi et al., 2013).

Most studies excluded patients with ESA exposure from four to eight weeks prior to study initiation or after prior ESA treatment for at least four weeks (Gotlib et al., 2009; Greenberg et al., 2009; Villegas et al., 2011; Kelaidi et al., 2013). Patients with previous ESA therapy were not accepted in the RCT of Balleari et al. (2006), whereas, Mannone et al. included patients with previous rHuEPO exposure (Mannone et al., 2006). No information concerning previous ESA therapy was provided in the other three trials (Nair et al., 2019; Hellstrom-Lindberg et al., 1998; Remacha et al., 1999). Three studies reported prior treatment with hematopoietic growth factors such as G-CSF as exclusion criterion (Balleari et al. (2006); Gotlib et al., 2009; Greenberg et al., 2009). Only two trials reported the Nordic MDS group score (Remacha et al., 1999; Gotlib et al., 2009), an acknowledged predictive model for erythroid response to treatment with ESA + G-CSF in anemic lower risk MDS patients (Hellstrom-Lindberg et al., 1997).

Relevant clinical characteristics and inclusion criteria remained unclear in the RCT conference abstract. Our efforts to request missing clinical and methodological data remained without success (Nair et al., 2019). Detailed patient characteristics can be found in Table 1 for RCTs and in Table 2 for clinical trials with SDAD.

3.4. Methodological characteristics of RCTs

RHuEPO was administered in all patients in a dose of 10,000 IU once weekly (Nair et al., 2019) and three times weekly respectively (Balleari et al., 2006) (Table 3). None of the RCTs reported any adjustment of rHuEPO doses according to hemoglobin levels. In the study of Balleari et al., patients were randomized in a 1:1 ratio and the experimental arm (50%) received G-CSF 300 mcg one to two times weekly additionally to rHuEPO, with dose adjustment of G-CSF according to neutrophil counts (Balleari et al., 2006). In the study of Nair et al., patients were randomized in a 4:1 ratio and the investigational arm (81%) received G-CSF 150 mcg every two days (Q2D) additionally to rHuEPO, without any G-CSF dose adjustment to neutrophil or white blood cell (WBC) counts (Nair et al., 2019). In the trial of Balleari et al., patients without erythroid response to rHuEPO alone after eight weeks had the possibility to cross-over to the experimental arm and take advantage of the combination therapy for additional eight weeks (Balleari et al., 2006). Cross-over was mentioned in the RCT of Nair et al. without providing more detailed information (Nair et al., 2019).

3.5. Methodological characteristics of clinical trials with SDAD

In the trials with SDAD, we focused on the additional erythroid response rates in patients receiving ESA + G-CSF (here phase 2 treatment) sequentially after minor or no response to ESA alone (here phase 1 treatment) (Table 4). During phase 1 treatment, four studies administered fix doses (Hellstrom-Lindberg et al., 1998; Mannone et al., 2006;

Table 3
Methods and results of RCTs.

Study *Author (Year)*	Patients *n (%)*	Treatment	Equivalent rHuEPO dose per week	Duration of treatment	Response criteria	Duration of response *months*	Outcome *n (%)*
(Balleari et al., 2006)	Arm A: 15/30 (50)	rHuEPO 10'000 IU 3x/week	30'000 IU	8 weeks	IWGc 2000	N/A	Erythroid response: 6/15 (40) Major response: 3/15 (20) Minor response: 3/15 (20)
	Non-responders in Arm A: 9/15 (60)	rHuEPO 10'000 IU 3x/week G-CSF 300 mcg 1-2x/week	30'000 IU	8 weeks			Erythroid response: 4/9 (44) Major response: 1/9 (11) Minor response: 3/9 (33)
	Arm B: 15/30 (50)	rHuEPO 10'000 IU 3x/week G-CSF 300 mcg 1-2x/week	30'000 IU	8 weeks			Erythroid response: 11/15 (73) Major response: 6/15 (40) Minor response: 5/15 (33)
(Nair et al., 2019)	Arm A: 13/68 (19)	rHuEPO 10'000 IU 1x/week	10'000 IU	12 weeks	Major response: Hb ≥ 115 g/L and transfusion-independency; Minor response: loss of transfusion-dependency	Median: 28 Range: 6-72	Erythroid response: 4/13 (33)
	Arm B: 55/68 (81)	rHuEPO 10'000 IU 1x/week G-CSF 150 mcg Q2D	10'000 IU				Erythroid response: 36/55 (65)

Abbreviations: G-CSF, granulocyte colony stimulating factor; Hb, hemoglobin; IWGc, International Working Group criteria; N/A, not available; Q2D, once every two days; rHuEPO, recombinant human erythropoietin.

Table 4
Methods and results of clinical trials with SDAD.

Study *Author (Year)*	Treatment in phase 1	Equivalent rHuEPO dose per week *	Duration of treatment in phase 1	Response criteria	Outcome after treatment in phase 1 *n (%)*	Requirements for entering phase 2	Treatment in phase 2	Duration of treatment in phase 2	Duration of response *months*	Outcome after treatment in phase 2 *n (%)*	Overall outcome after phase 2 *n (%)*
(Hellstrom-Lindberg et al., 1998)	rHuEPO 5'000 or 10'000 IU Q1D (dose escalation after 2 weeks if necessary)	35'000 IU or 70'000 IU	8 weeks	Major response: Hb ≥ 115 g/L Minor response: increase in Hb ≥ 15 g/L or loss of transfusion-dependency and stable Hb for ≥ 4 weeks	Erythroid response: 3/26 (12) Major response: 1/26 (4) Minor response: 2/26 (8) No response: 23/26 (88) Evaluable after phase 2: 20/23 (87)	No response	rHuEPO 5'000 or 10'000 IU Q1D G-CSF 30 or 75 or 150 mcg Q1D (dose escalation after every 2 weeks if necessary)	10 weeks	Median: 24 Range: 4-60	Erythroid response: 6/20 (30) Major response: 3/20 (15) Minor response: 3/20 (15)	Erythroid response: 9/26 (35) Major response: 4/26 (16) Minor response: 5/26 (19)
(Remacha et al., 1999)	rHuEPO 300 IU/kg 3x/week	63'000 IU	8 weeks	IWGc 2000	Erythroid response: 11/32 (34) Major response: 7/32 (22) Minor response: 4/32 (12) No response: 21/32 (66) Evaluable after phase 2: 14/25 (56)	No response Minor response	rHuEPO 300 IU/kg 3x/week G-CSF 1 mcg/kg 3x/week	8 weeks	N/A	Erythroid response: 7/14 (50) Major response: 3/14 (21) Minor response: 4/14 (29)	Erythroid response: 16/32 (50) Major response: 12/32 (38) Minor response: 4/32 (12)
(Mannone et al., 2006)	Darbepoetin 300 mcg Q1W	60'000 IU	12 weeks	IWGc 2000	Erythroid response: 44/62 (71) Major response: 34/62 (55) Minor response: 10/62 (16) No response: 18/62 (29) Evaluable after phase 2: 10/18 (56)	No response	Darbepoetin 300 mcg Q1W Filgrastim 300 mcg or Lenograstim 105 mcg 3x/week	12 weeks	Median: 10 Range: 1-21	Erythroid response: 2/10 (20) Major response: 1/10 (10) Minor response: 1/10 (10)	Erythroid response: 46/62 (74) Major response: 35/62 (56) Minor response: 11/62 (18)
(Gotlib et al., 2009)	Darbepoetin 4.5 mcg/kg or 9 mcg/kg Q1W (dose escalation after 6 weeks if necessary; RARS-patients start with Darbepoetin 9 mcg/kg Q1W)	63'000 IU or 126'000 IU	12 weeks	IWGc 2000	Erythroid response: 6/24 (25) Major response: 5/24 (21) Minor response: 1/24 (4) No response: 18/24 (75) Evaluable after phase 2: 15/19 (79)	No response Minor response	Darbepoetin 9 mcg/kg Q1W G-CSF 2.5 ± 1 mcg/kg 2x/week	6 weeks	Major responders: Median: 12 Range: 4-19 Minor responders: Median: 5 Range: 2-7.5	Erythroid response: 10/15 (67) Major response: 7/15 (47) Minor response: 3/15 (20)	Erythroid response: 16/24 (67) Major response: 12/24 (50) Minor response: 4/24 (17)
(Greenberg et al., 2009)	rHuEPO 150 IU/kg Q1D	73'500 IU	16 weeks	IWGc 2000 (response for at least 4 months instead of 2 months)	Erythroid response: 25/73 (34) Major response: N/A Minor response: N/A No response: 48/73 (66) Evaluable after phase 2: 27/48 (56)	No response	rHuEPO 150 IU/kg Q1D G-CSF 1 mcg/kg Q1D Without response: rHuEPO 300 IU/kg Q1D G-CSF 1 mcg/kg Q1D	N/A	N/A	Erythroid response: 9/27 (33) rHuEPO 150 IU/kg Q1D: Erythroid response: 5/27 (19) rHuEPO 300 IU/kg Q1D: Erythroid response: 4/12 (33)	Erythroid response: 34/73 (47) Major response: N/A Minor response: N/A
(Villegas et al., 2011)	Darbepoetin 300 mcg Q1W	60'000 IU	8 weeks	IWGc 2000	Erythroid response: 31/44 (70) Major response: 21/44 (47) Minor response: 10/44 (23) No response: 13/44 (30) Evaluable after phase 2: 18/23 (78)	No response Minor response	Darbepoetin 300 mcg Q1W G-CSF 300 mcg Q1W	8 weeks	N/A	Erythroid response: 6/18 (33) Major response: 6/18 (33) Minor response: 0/18 (0)	Erythroid response: 31/44 (70) Major response: 27/44 (61) Minor response: 4/44 (9)

(*continued on next page*)

Table 4 (*continued*)

Study *Author (Year)*	Treatment in phase 1	Equivalent rHuEPO dose per week *	Duration of treatment in phase 1	Response criteria	Outcome after treatment in phase 1 *n (%)*	Requirements for entering phase 2	Treatment in phase 2	Duration of treatment in phase 2	Duration of response *months*	Outcome after treatment in phase 2 *n (%)*	Overall outcome after phase 2 *n (%)*
(Kelaidi et al., 2013)	Darbepoetin 500 mcg Q2W	50'000 IU	12 weeks	IWGc 2006	Erythroid response: 46/95 (48) No response: 49/95 (52) Evaluable after phase 2: 40/49 (82)	No response	Darbepoetin 500 mcg Q2W G-CSF 300 mcg 2x/week	12 weeks	Median: RARS: 27.3 RCMD: 56 RCMD-RS: 19.8 RAEB-1: 20.6	Erythroid response: 9/40 (22)	Erythroid response: 55/95 (58)

* 1 mcg Darbepoetin approximately corresponds to rHuEPO 200 IU; 70 kg standard body weight was assumed. Abbreviations: G-CSF, granulocyte colony stimulating factor; Hb, hemoglobin; IWGc, International Working Group criteria; N/A, not available; Q1D, once daily; Q1W, once weekly; Q2W, once every two weeks; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenias with multilineage dysplasia; RCMD-RS, refractory cytopenias with multilineage dysplasia and ring sideroblasts; rHuEPO, recombinant human erythropoietin.

Villegas et al., 2011; Kelaidi et al., 2013) and three studies weight-based doses of ESA (Remacha et al., 1999; Gotlib et al., 2009; Greenberg et al., 2009). The total administered doses of rHuEPO and darbepoetin ranged from 35,000 to 126,000 IU and from 300 to 630 mcg per week, respectively. The frequency of application ranged from once daily to once every two weeks (Q2W). In five of seven studies, the frequency of ESA application was decreased, if hemoglobin levels exceed 120 g/L (Villegas et al., 2011; Kelaidi et al., 2013), 130 g/L (Gotlib et al., 2009; Greenberg et al., 2009) or 140 g/L (Mannone et al., 2006). A dose escalation of ESA was reported by Gotlib et al. after six weeks, if patients did not achieve a hemoglobin rise of > 20 g/L (Gotlib et al., 2009). Another trial reported dose escalation of ESA after two weeks if necessary, without describing more details (Hellstrom-Lindberg et al., 1998). The duration of phase 1 treatment ranged from eight to sixteen weeks.

Requirements for entering phase 2 treatment were no erythroid response after phase 1 treatment in four studies (Hellstrom-Lindberg et al., 1998; Mannone et al., 2006; Greenberg et al., 2009; Kelaidi et al., 2013) and minor or no erythroid response in three studies according to IWGc or similar criteria (see below) (Remacha et al., 1999; Gotlib et al., 2009; Villegas et al., 2011). Treatment during phase 2 consisted of ESA dosage continued as in phase 1 with the addition of G-CSF in fix doses in four studies (Hellstrom-Lindberg et al., 1998; Mannone et al., 2006; Villegas et al., 2011; Kelaidi et al., 2013) and in weight-adjusted doses in three studies (Remacha et al., 1999; Gotlib et al., 2009; Greenberg et al., 2009). The G-CSF doses ranged from 210 to 1,050 mcg weekly and the frequency of application ranged from daily to once weekly (Q1W). In five trials, the G-CSF dosage was adjusted to either WBC or absolute neutrophil counts (Mannone et al., 2006; Gotlib et al., 2009; Greenberg et al., 2009; Villegas et al., 2011; Kelaidi et al., 2013), whereas one trial reported the possibility of a dose escalation Q2W, if necessary (Hellstrom-Lindberg et al., 1998). Greenberg et al. reported a doubling of ESA dosage in phase 2 treatment in some patients (Greenberg et al., 2009). Evaluation of erythroid response was done between six and twelve weeks after start of treatment in phase 2 in all studies except one, in which duration of phase 2 was not reported (Greenberg et al., 2009).

3.6. Outcomes

Seven out of nine studies reported erythroid response according to IWGc; six studies (Balleari et al., 2006; Remacha et al., 1999; Mannone et al., 2006; Gotlib et al., 2009; Greenberg et al., 2009; Villegas et al., 2011) used the IWGc published in 2000 (Cheson et al., 2000) and one study (Kelaidi et al., 2013) the revised IWGc from 2006 (Cheson et al., 2006). The criteria for erythroid response reported by the two remaining records were based on changes in baseline hemoglobin levels and proportion of transfusion-dependency, similar to the IWGc, which therefore met our eligibility criteria (Nair et al., 2019; Hellstrom-Lindberg et al., 1998).

The RCT of Balleari et al. reported erythroid response rates of 73% (11/15 patients) in patients receiving rHuEPO + G-CSF compared to 40% (6/15 patients) receiving rHuEPO alone after eight weeks, resulting in a relative risk (RR) of 1.833 (95% CI 0.919–3.658) favoring rHuEPO + G-CSF (Fig. 2 and Table 3). Nine non-responders to rHuEPO crossed over to the experimental arm and received combination therapy for another eight weeks, resulting in 44% (4/9 patients) with an erythroid response after 16 weeks. Overall erythroid response after 16 weeks (including cross-over) was 63% (15/24 patients) in patients receiving rHuEPO + G-CSF compared to 33% (5/15 patients) in the arm with rHuEPO alone (Balleari et al., 2006). Nair et al. evaluated their patients after twelve weeks of treatment and described erythroid response in 65% (36/55 patients) in the experimental arm with rHuEPO + G-CSF compared to 33% (4/13 patients) in patients with rHuEPO alone, resulting in a RR of 2.127 (95% CI 0.920–4.916) favoring rHuEPO + G-CSF (Nair et al., 2019) (Fig. 2 and Table 3). Combined RR calculated with a random effects model was 1.947 (95% CI 1.143–3.318) favoring rHuEPO + G-CSF for both studies.

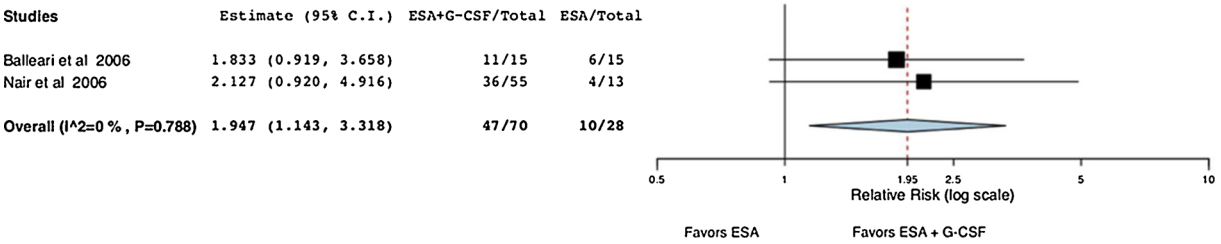

Fig. 2. Forest plot showing risk ratios and 95% CI of erythroid response rates of included RCTs after treatment with ESA + G-CSF compared to ESA alone. Abbreviations: ESA, erythropoiesis stimulating agents; G-CSF, granulocyte colony stimulating factor; 95% C.I., 95% confidence interval.

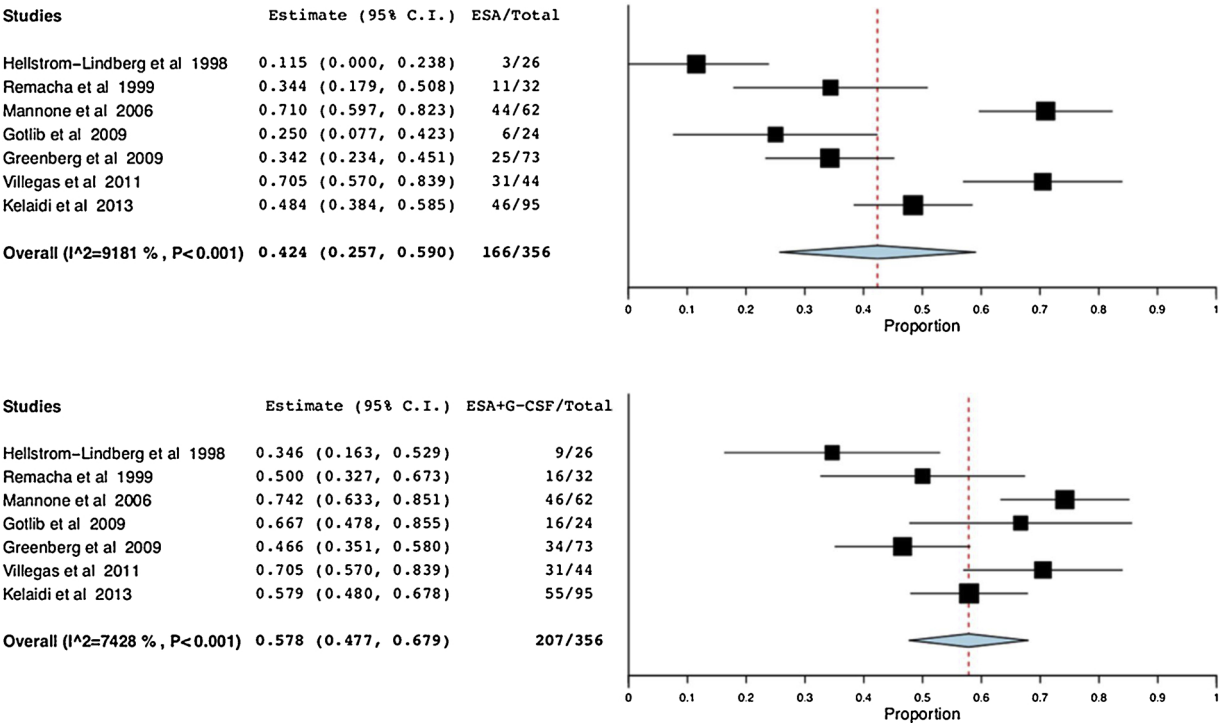

Fig. 3. Proportions and 95% CI of erythroid responses of included clinical trials with SDAD after treatment with ESA alone and ESA + G-CSF. Abbreviations: ESA, erythropoiesis stimulating agents; G-CSF, granulocyte colony stimulating factor; 95% C.I., 95% confidence interval.

The overall erythroid response rates in the seven trials with SDAD ranged from 12% to 71% after phase 1 (ESA only) and from 35% to 74% after phase 2 (ESA + G-CSF). Thus, erythroid response rates increased between 0% to 42% during phase 2 treatment (Fig. 3 and Table 4). Patients showing minor or no response after phase 1 got erythroid responders in 20% to 67% after the combination therapy in phase 2. Major erythroid response rates after phase 1 ranged from 4% to 55%, whereas major erythroid responders ranged from 16% to 61% after phase 2 in five studies (Hellstrom-Lindberg et al., 1998; Remacha et al., 1999; Mannone et al., 2006; Gotlib et al., 2009; Villegas et al., 2011). Response criteria in one trial did not distinguish between major and minor erythroid response after phase 2 (Greenberg et al., 2009). In this trial, four out of twelve patients showed erythroid response after doubling the rHuEPO dosage in phase 2 (Greenberg et al., 2009). Kelaidi et al. reported erythroid response rates based on revised IWGc and therefore did not distinguish between major and minor erythroid responses (Kelaidi et al., 2013).

In most studies, time to response, duration of response, progression to higher risk MDS or AML as well as adverse effects were mentioned for all patients in general, but not for the distinct treatment groups and impeded the investigation of our predefined secondary endpoints.

3.7. Risk of bias

The corresponding publications did not provide sufficiently detailed information on study designs, therefore, risk of bias of the selected RCTs and trials with SDAD remained unclear in most cases (Supplementary tables 3–4).

4. Discussion

In this systematic review we screened the currently available medical literature for randomized and non-randomized trials investigating the additional effect of ESA + G-CSF compared to ESA alone on erythroid response in anemic lower risk MDS patients. We identified only two RCTs that were appropriately designed for our study question. Therefore, we extended our analysis to non-randomized trials with SDAD, of which only seven fulfilled our inclusion criteria. The aggregated erythroid response rates from RCTs ranged from 65% to 73% for ESA + G-CSF compared to 33% to 40% for ESA monotherapies. The trials with SDAD reported erythroid response rates ranging from 35% to 74% for ESA + G-CSF compared to 12% to 71% for ESA monotherapies. Overall, most studies suggested that the addition of G-CSF to ESA

improved erythroid response in anemic lower risk MDS patients compared to ESA alone. However, low patient numbers, inappropriate design of the studies, relevant differences in drug schedules and variable time-points for assessment of endpoints prevented the performance of a proper meta-analysis. Due to limited information on study designs, many additional sources for potential biases remain unclear, which additionally limit clinical evidence and firm conclusions.

To our knowledge, this is the first systematic review investigating the additional efficacy of ESA + G-CSF compared to ESA alone in the treatment of anemic lower risk MDS patients. Nevertheless, several limitations need to be mentioned. Firstly, the scarcity of data derived from properly designed RCTs was unexpected and from 1,287 screened records, only nine publications met our extended eligibility criteria. The two RCTs encompassed less than 100 patients, of which two thirds were reported in an abstract with unpublished data and incomplete reporting (Nair et al., 2019). Both studies showed an additional benefit concerning erythroid response rates for the combination therapy compared to ESA alone. However, the administration of rHuEPO 10,000 IU once to three times weekly for a maximal duration of eight weeks, as used in the two reported RCTs (Balleari et al., 2006; Nair et al., 2019), must nowadays be considered to be insufficient. Full-dose ESA of up to 60,000-80,000 IU rHuEPO equivalent per week for a maximum duration of twelve weeks are recommended in current guidelines and should have been used instead (Malcovati et al., 2013; Greenberg et al., 2017).

The heterogeneity of study designs of the additional seven trials with SDAD challenge the robust interpretation of erythroid response rates. The clinical trials with SDAD are not ideal to investigate the additional efficacy of combination treatment, because distinction between the additional effect of sequential combination with G-CSF and late erythroid responses on extended ESA monotherapy is not reliably possible. This accounts especially for those studies, in which ESA was not administered in full-dose (60,000-80,000 IU rHuEPO equivalent per week) and for sufficient time (twelve weeks) during phase 1 treatment. Even the observation that patients lost responses after withdrawal of G-CSF does not exclude the intrinsic natural course with loss of responses to ESA, independent of concomitant use of G-CSF. The erythroid response to ESA is generally expected to occur between six and twelve weeks (Greenberg et al., 2017; Santini, 2016). However, late responses after prolonged ESA administration have been reported. As an example, Terpos et al. showed overall erythroid response rates of 18% after twelve weeks and 45% after 26 weeks of ESA exposure (Terpos et al., 2002). Therefore, insufficient dosing and exposure time to ESA in studies with SDAD have potentially biased the currently accepted notion that combination treatment provides additional benefit on erythroid response compared to single ESA treatment.

The evaluation of our predefined secondary endpoints composting time to response, duration of response, progression to higher risk MDS or AML, quality of life and side effects was not possible, as this information was mainly reported for the whole patient population and not for the corresponding subgroups. Therefore, safety issues concerning progression to higher risk MDS and AML caused by continuous administration of G-CSF, in analogy to the potential long-term side-effects of continuous application of thrombopoietin-stimulating agents, remain a concern that cannot be faithfully excluded (Prica et al., 2014; Dodillet et al., 2017). An RCT investigating the effect of ESA + G-CSF versus supportive care in lower risk MDS patients reported erythroid response rates of 42% (10/24 patients) in the therapy arm compared to 0% (0/26 patients) in the supportive care arm (consisting of RBC transfusions and iron-chelation treatment) after 12 weeks (Casadevall et al., 2004). Meta-analyses investigating the effect of ESA alone in the treatment of anemia in lower risk MDS patients showed estimated pooled erythroid response rates of 44% in patients treated with epoetin alpha and erythroid response rates of 38% to 72% in patients treated with darbepoetin alpha (Moyo et al., 2008; Park et al., 2016). Overall, these studies demonstrate that also indirect comparisons from studies investigating ESA + G-CSF versus supportive care compared to ESA alone versus supportive care do not provide convincing evidence for an increased erythroid response rate in anemic lower risk MDS patients. The synergistic effect of G-CSF might be only limited in those conditions, where ESA signaling is not saturated.

We conclude that the number of clinical trials is low, the sizes of study populations small and the study designs not fully appropriate for a strong recommendation that ESA + G-CSF provide additional efficacy compared to ESA monotherapy in the treatment of anemic lower risk MDS patients. Based on our systematic review, the statement of current guidelines that treatment with ESA + G-CSF is based on Ib evidence with a grade A recommendation is formally correct but overestimates the available evidence (Malcovati et al., 2013; Shekelle et al., 1999; Harbour and Miller, 2001). Other recently published reviews, including a Cochrane database review, support the lack of data from clinical trials and especially the small numbers of RCTs as a major issue (Santini, 2016; Hutzschenreuter et al., 2016). Therefore, RCTs investigating the effect of ESA + G-CSF compared to ESA alone, especially using full-doses of ESA (60,000-80,000 IU rHuEPO equivalent per week) for a sufficient time (at least twelve weeks), would be desirable to assess indisputably the additional benefit of the combination treatment. Moreover, the required studies should also investigate duration of response, quality of life as well as safety endpoints including progression to higher risk MDS or AML and adverse effects. Based on the new insights in the pathogenesis of MDS gained by genetic profiling and stem cell research, it will be difficult to launch such appropriately designed RCT for a treatment that would fit all. Instead, the current research focusses more on personalized prediction to identify those patients that profit most from a specific intervention. Properly randomized and designed clinical trials with appropriate dose and duration of ESA treatment would be generally required but not attractive in the current era of “precision medicine”. Standardizing ESA treatment and definition of refractoriness (dose and duration) will help to identify those patients that are candidates for future clinical trials with novel compounds.

Our systematic review supports an additional benefit of ESA + G-CSF compared to low- and standard-dose ESA in anemic lower risk MDS patients. However, the clinical evidence for an additional benefit of G-CSF over full-dose ESA alone is based on inadequately designed studies, and, therefore, is not sufficient for a strong recommendation. Compared to large randomized controlled trials, evidence from these early studies are not very strong and current guidelines should mention these limitations.

Author’s contributions

LA: performed systematic study selection, extracted data, analyzed data and wrote the paper; JB: designed the study, reviewed the selected studies, analyzed data and critically reviewed the paper; MH: performed statistical analysis and critically reviewed the paper; NB: initiated and designed the study, reviewed the selected studies, verified data extraction, analyzed data and wrote the paper.

Trial registration

Not required.

Funding

None.

Conflict ofinterest

The authors declare no conflict of interest for this study.

Acknowledgement

The authors would like to thank Professor Eva Hellström-Lindberg for her critical review of the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.critrevonc.2019.01.021.

References

Balleari, E., Rossi, E., Clavio, M., et al., 2006. Erythropoietin plus granulocyte colony-stimulating factor is better than erythropoietin alone to treat anemia in low-risk myelodysplastic syndromes: results from a randomized single-centre study. Ann. Hematol. 85 (3), 174–180.
Bernell, P., Stenke, L., Wallvik, J., Hippe, E., Hast, R., 1996. A sequential erythropoietin and GM-CSF schedule offers clinical benefits in the treatment of anaemia in myelodysplastic syndromes. Leuk. Res. 20 (8), 693–699.
Bonadies, N., Feller, A., Rovo, A., et al., 2017. Trends of classification, incidence, mortality, and survival of MDS patients in Switzerland between 2001 and 2012. Cancer Epidemiol. 46, 85–92.
Casadevall, N., Durieux, P., Dubois, S., et al., 2004. Health, economic, and quality-of-life effects of erythropoietin and granulocyte colony-stimulating factor for the treatment of myelodysplastic syndromes: a randomized, controlled trial. Blood 104 (2), 321–327.
Chen, T.L., Chiang, Y.W., Lin, G.L., et al., 2018. Different effects of granulocyte colony-stimulating factor and erythropoietin on erythropoiesis. Stem Cell Res. Ther. 9 (1), 119.
Cheson, B.D., Bennett, J.M., Kantarjian, H., et al., 2000. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood 96 (12), 3671–3674.
Cheson, B.D., Greenberg, P.L., Bennett, J.M., et al., 2006. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 108 (2), 419–425.
Cogle, C.R., 2015. Incidence and burden of the myelodysplastic syndromes. Curr. Hematol. Malig. Rep. 10 (3), 272–281.
Dodillet, H., Kreuzer, K.A., Monsef, I., Skoetz, N., 2017. Thrombopoietin mimetics for patients with myelodysplastic syndromes. Cochrane Database Syst. Rev. 9, Cd009883.
Fenaux, P., Mufti, G.J., Hellstrom-Lindberg, E., et al., 2009. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 10 (3), 223–232.
Gotlib, J., Lavori, P., Quesada, S., Stein, R.S., Shahnia, S., Greenberg, P.L., 2009. A Phase II intra-patient dose-escalation trial of weight-based darbepoetin alfa with or without granulocyte-colony stimulating factor in myelodysplastic syndromes. Am. J. Hematol. 84 (1), 15–20.
Greenberg, P., Cox, C., LeBeau, M.M., et al., 1997. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood 89 (6), 2079–2088.
Greenberg, P.L., Sun, Z., Miller, K.B., et al., 2009. Treatment of myelodysplastic syndrome patients with erythropoietin with or without granulocyte colony-stimulating factor: results of a prospective randomized phase 3 trial by the Eastern Cooperative Oncology Group (E1996). Blood 114 (12), 2393–2400.
Greenberg, P.L., Tuechler, H., Schanz, J., et al., 2012. Revised international prognostic scoring system for myelodysplastic syndromes. Blood 120 (12), 2454–2465.
Greenberg, P.L., Stone, R.M., Al-Kali, A., et al., 2017. Myelodysplastic syndromes, version 2.2017, NCCN clinical practice guidelines in oncology. J. Natl. Comp. Cancer Netw.: JNCCN 15 (1), 60–87.
Harbour, R., Miller, J., 2001. A new system for grading recommendations in evidence based guidelines. BMJ 323 (7308), 334–336.
Hellstrom-Lindberg, E., Negrin, R., Stein, R., et al., 1997. Erythroid response to treatment with G-CSF plus erythropoietin for the anaemia of patients with myelodysplastic syndromes: proposal for a predictive model. Br. J. Haematol. 99 (2), 344–351.
Hellstrom-Lindberg, E., Ahlgren, T., Beguin, Y., et al., 1998. Treatment of anemia in myelodysplastic syndromes with granulocyte colony-stimulating factor plus erythropoietin: results from a randomized phase II study and long-term follow-up of 71 patients. Blood 92 (1), 68–75.
Higgins, J.P., Altman, D.G., Gotzsche, P.C., et al., 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 343, d5928.
Hogge, D.E., Cashman, J.D., Humphries, R.K., Eaves, C.J., 1991. Differential and synergistic effects of human granulocyte-macrophage colony-stimulating factor and human granulocyte colony-stimulating factor on hematopoiesis in human long-term marrow cultures. Blood 77 (3), 493–499.
Hutzschenreuter, F., Monsef, I., Kreuzer, K.A., Engert, A., Skoetz, N., 2016. Granulocyte and granulocyte-macrophage colony stimulating factors for newly diagnosed patients with myelodysplastic syndromes. Cochrane Database Syst. Rev. 2, Cd009310.
Jaffe, E.S., Harris, N.L., Stein, H., Vardiman, J.W. (Eds.), 2001. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon, France.
Kelaidi, C., Beyne-Rauzy, O., Braun, T., et al., 2013. High response rate and improved exercise capacity and quality of life with a new regimen of darbepoetin alfa with or without filgrastim in lower-risk myelodysplastic syndromes: a phase II study by the GFM. Ann. Hematol. 92 (5), 621–631.
Malcovati, L., Hellstrom-Lindberg, E., Bowen, D., et al., 2013. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood 122 (17), 2943–2964.
Mannone, L., Gardin, C., Quarre, M.C., et al., 2006. High-dose darbepoetin alpha in the treatment of anaemia of lower risk myelodysplastic syndrome results of a phase II study. Br. J. Haematol. 133 (5), 513–519.
Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J. Clin. Epidemiol. 62 (10), 1006–1012.
Moyo, V., Lefebvre, P., Duh, M.S., Yektashenas, B., Mundle, S., 2008. Erythropoiesis-stimulating agents in the treatment of anemia in myelodysplastic syndromes: a meta-analysis. Ann. Hematol. 87 (7), 527–536.
Nair, V., Mishra, D., Sharma, A., et al., 2006. Erythropoetin (EPO) and granulocyte colony stimulating factor (GCS-F) based therapy in patients with low risk MDS: a single centre experience from India. Blood 303.
Park, S., Fenaux, P., Greenberg, P., et al., 2016. Efficacy and safety of darbepoetin alpha in patients with myelodysplastic syndromes: a systematic review and meta-analysis. Br. J. Haematol. 174 (5), 730–747.
Prica, A., Sholzberg, M., Buckstein, R., 2014. Safety and efficacy of thrombopoietin-receptor agonists in myelodysplastic syndromes: a systematic review and meta-analysis of randomized controlled trials. Br. J. Haematol. 167 (5), 626–638.
Remacha, A.F., Arrizabalaga, B., Villegas, A., et al., 1999. Erythropoietin plus granulocyte colony-stimulating factor in the treatment of myelodysplastic syndromes. Identification of a subgroup of responders. The Spanish Erythropathology Group. Haematologica 84 (12), 1058–1064.
Santini, V., 2016. Treatment of low-risk myelodysplastic syndromes. Hematology Am. Soc. Hematol. Educ. Program 2016 (1), 462–469.
Shekelle, P.G., Woolf, S.H., Eccles, M., Grimshaw, J., 1999. Clinical guidelines: developing guidelines. BMJ 318 (7183), 593–596.
Steensma, D.P., 2015. Myelodysplastic syndromes: diagnosis and treatment. Mayo Clin. Proc. 90 (7), 969–983.
Sterne, J.A., Hernan, M.A., Reeves, B.C., et al., 2016. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ 355, i4919.
Swerdlow, S.H., Campo, E., Harris, N.L., et al., 2008. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. IARC Press, Lyon, France.
Swerdlow, S.H., Campo, E., Harris, N.L., et al., 2016. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, revised 4th ed. IARC Press, Lyon, France.
Tehranchi, R., Fadeel, B., Schmidt-Mende, J., et al., 2005. Antiapoptotic role of growth factors in the myelodysplastic syndromes: concordance between in vitro and in vivo observations. Clin. Cancer Res. 11 (17), 6291–6299.
Terpos, E., Mougiou, A., Kouraklis, A., et al., 2002. Prolonged administration of erythropoietin increases erythroid response rate in myelodysplastic syndromes: a phase II trial in 281 patients. Br. J. Haematol. 118 (1), 174–180.
Villegas, A., Arrizabalaga, B., Fernandez-Lago, C., et al., 2011. Darbepoetin alfa for anemia in patients with low or intermediate-1 risk myelodysplastic syndromes and positive predictive factors of response. Curr. Med. Res. Opin. 27 (5), 951–960.

Lucas Affentranger (LA) studied at the Medical Faculty of the University of Berne and obtained his Master of Medicine along with the Federal Diploma in Medicine in 2018. His first contact with MDS research was during his work as a datamanager for the Swiss MDS Study Group. With the current systematic review he aims for gaining the doctoral degree in Medicine. Mr Affentranger is currently working as a medical intern at the Swiss Paraplegic Centre in Nottwil, Switzerland.

PD Dr. med. Julia Bohlius (JB) studied medicine at the University of Hamburg from 1992 to 1999, with internships and residencies in Colombia, China, USA and Germany. In 2002 she obtained her doctorate at the Department of Hematology, Oncology and Infectious Diseases at the University of Cologne with a systematic literature review on the treatment of lymphoma. Two years later she received her Master of Science in Public Health from the London School of Hygiene and Tropical Medicine. Back in Cologne, she worked at the Department of Hematology, Oncology and Infectious Diseases on the one hand and as a research associate at the Cochrane Hematological Malignancies Group. In 2007 she came to Switzerland to the Institute for Social and Preventive Medicine at the University of Berne, where she heads the Cancer Research Group. She specializes in systematic reviews of clinical cancer research studies and meta-analyses based on large datasets of individual clinical patient data.

Mahmoud Hallal (MH) obtained a bachelor of science in Biology at the University of Neuchâtel, Switzerland in 2014. Then he completed his Master degree in Bioinformatics and Computational Biology at the University of Bern in 2016, where his master thesis concentrated on applying machine learning methods on microarray expression data to predict clinical variables. Actually, he is doing his PhD thesis at the department of Hematology of the University Hospital of Bern (Inselspital) concentrating on proteomics analysis in MDS and AML diseases and supports statistical analyses at the laboratory of PD Dr. med. Nicolas Bonadies.

PD Dr. med. Nicolas Bonadies (NB) studied medicine at the University of Bern from 1992 to 1998 and obtained the degree of medical doctor in 2002. He received his board certification as specialist in Internal Medicine in 2005, Clinical Hematology in 2012 and Hematological Laboratory Analyses in 2014. During his post-doctoral fellowship at Cambridge University, UK, he investigated gene-regulation in hematological neoplasms from 2007-2009. He is currently a senior attending physician in the Clinic of Hematology and Hematological Laboratory of the Inselspital Bern in Switzerland and holding a tenure track position as independent group leader for the investigation of myeloid neoplasms since 2016. PD Dr. Bonadies was appointed the position of director of the MDS Centre of Excellence. He is also directing the research unit, patient registry/biobank and is associate director of the clinical hematological services at the Inselspital Bern. Moreover, he is also chairing the Swiss MDS Study Group with a Swiss MDS Registry and Biobank and is a member of the Leukaemia Group of the Swiss Group of Clinical Cancer Research (SAKK).